

34. (Amended) A nucleic acid coding for an essential secretory gene for Helicobacter, identified by the method as claimed in claim 22.

35. (Amended) A gene library comprising at least two nucleic acids claimed in claim 34.

44. (Amended) A polypeptide characterized in that it is encoded by nucleic acid as claimed in claim 34.

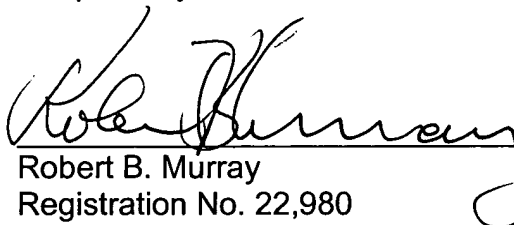
50. (Amended) The use of a polypeptide or of a fragment thereof as claimed in claim 44 thereof as immunogen for generating antibodies.

#### REMARKS

Claims 1-58 are pending in this application. By this Amendment, claims 4,5, 8-12, 15-19, 21, 29, 32, 33, 34, 38, 39, 44, 50 are amended to correct the multiple dependency thereof and to place this application into better condition for examination.

No new matter is added.

Respectfully submitted,

  
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MARKED UP CLAIMS

- 93 -

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### Claims

1. A method for providing agents for the detection, for the prevention or/and for the therapy of microbial infections,  
5 **characterized in that**  
it comprises the steps:
- (A) identification of essential genes and the corresponding polypeptides  
by producing gene-deficient microorganisms by conditional  
10 antisense inhibition (CAI) or/and subtractive recombination  
mutagenesis (SRM) and determining the viability or/and survivability  
of the gene-deficient microorganisms in an assay system.
- (B) identification of specific active ingredients which are directed  
15 against the essential polypeptides and bring about inactivation of the  
microorganisms or used microorganisms.
- (C) testing of the identified active ingredients for their usability as  
components of diagnostic, preventive or/and therapeutic  
compositions,
- (D) formulation of the useful active ingredients as diagnostic, preventive  
20 or/and therapeutic compositions.
2. A method as claimed in claim 1,  
**characterized in that**  
obligately essential genes are identified by CAI.
- 25 3. A method as claimed in claim 1,  
**characterized in that**  
facultatively essential genes are identified by SRM.
- 30 4. A method as claimed in ~~any of the preceding claims,~~  
*claim 1*  
**characterized in that**

step (A) is preceded by selection for genes which code for polypeptides having a particular functionality or/and which code for polypeptides which are expressed in a particular stage of development.

5      5.      *claim 1*  
A method as claimed in ~~any of the preceding claims~~,  
characterized in that  
the selection is carried out with the aid of hybridization methods selected from subtraction and array methods.

10      6.      A method as claimed in claim 5,  
characterized in that  
the selection is carried out for specific subtracted apathogenic or pathogenic genes.

15      7.      A method as claimed in claim 5,  
characterized in that  
the selection is carried out for specific subtracted genes of *H. pylori* or *H. heilmannii*.

20      8.      *claim 4*  
A method as claimed in ~~any of claims 4 to 7~~,  
characterized in that  
gene sequences coding for exported polypeptides are selected.

25      9.      *claim 4*  
A method as claimed in ~~any of claims 4 to 8~~,  
characterized in that  
gene sequences coding for secreted polypeptides are selected.

30      10.      *claim 4*  
A method as claimed in ~~any of claims 4 to 9~~,  
characterized in that  
genes which code for polypeptides and which are necessary for the development of the vital form from the resistant form are selected.

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11. A method as claim d in ~~any of claims 4 to 9,~~  
characterized in that *claim 4,*  
genes which code for polypeptides and which are necessary for development  
of the resistant form from the vital form are selected.

5  
12. A method as claimed in ~~any of the preceding claims,~~  
characterized in that *claim 1,*  
in step (A) test systems selected from *in-vitro* systems, cell culture systems,  
tissue culture systems and animal models are used as natural environment  
10 for determining the viability and survivability of the gene-deficient  
microorganism.

13. A method as claimed in claim 12,  
characterized in that  
15 the deficient gene sequences which lead to gene-deficient microorganisms  
which are not culturable and incapable of survival in the natural environment  
are assigned to the category of obligately essential genes.

14. A method as claimed in claim 12,  
characterized in that  
20 the deficient gene sequences which lead to gene-deficient microorganisms  
which are culturable but incapable of survival in the natural environment are  
assigned to the category of facultatively essential genes.

25 15. A method as claimed in ~~any of the preceding claims,~~  
characterized in that *claim 1,*  
the identified genes are used to produce primers with whose aid  
corresponding genes from related microorganisms, subspecies or/and  
species are identified.

30 16. A method as claimed in ~~any of the preceding claims,~~  
characterized in that *claim 1,*

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in step(B) there is identification of specific active ingredients which influence the expression, presentation and/or function of the essential polypeptides, in particular immunologically active substances, binding partners of the polypeptides or fragments thereof or/and inhibitory substances.

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17. A method as claimed in <sup>claim 1</sup> ~~any of the preceding claims~~,  
**characterized in that**

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step(B) comprises a determination of the immunogenic potential of the polypeptides or/and fragments thereof, with the identified genes being expressed and subsequently a Western blot analysis being carried out or/and in that the identified polypeptides or fragments thereof are used to carry out a vaccination in cell culture or in an animal model, and the induction of a specific immune response is observed.

15

18. A method as claimed in <sup>claim 1</sup> ~~any of the preceding claims~~,  
**characterized in that**

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step (B) comprises a determination of the binding potential of the polypeptides or fragments thereof by screening of substance libraries, surface display methods, crystallographic analysis or/and computer modelling.

25

19. A method as claimed in <sup>claim 1</sup> ~~any of the preceding claims~~,  
**characterized in that**

the diagnostic, preventive or/and therapeutic agents are provided in the form of passive vaccines or active vaccines.

20. A method as claimed in claim 19,  
**characterized in that**

30

the passive vaccines are provided in the form of antibodies or/and antibody fragments and the active vaccines are provided in the form of heterologous carrier systems or/and in the form of antigens or antigen fragments, subunit vaccines, live vaccines, DNA vaccines or/and food vaccines.

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21. A method as claimed in ~~any of the preceding claims 1 to 19,~~  
*Claim 1*  
**characterized in that**

the diagnostic, preventive or/and therapeutic agents comprise inhibitory substances, in particular expression inhibitors or/and enzyme inhibitors.

5

22. A method for identifying essential microbial genes,  
**characterized in that**

it comprises the steps:

- (i) production of gene-deficient microorganisms,
- 10 (ii) determination of the viability or/and survivability of the gene-deficient microorganisms from (i),
- (iii) identification of a protein-encoding section of a microbial DNA sequence in which the gene-deficient microorganisms are deficient.
- (iv) Characterization of those DNA sections which are essential for survivability.

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23. A method as claimed in claim 22,  
**characterized in that**

the gene-deficient microorganisms are produced by mutagenizing a DNA section in a microbial genome.

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24. A method as claimed in claim 22,  
**characterized in that**

the DNA section is mutagenized by transposon mutagenesis.

25

25. A method as claimed in claim 23,  
**characterized in that**

the mutagenization of the DNA section on the microbial genome takes place by homologous recombination.

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26. A method as claimed in claim 25,  
**characterized in that**

the SRM method is used.

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27. A method as claimed in claim 23,  
characterized in that  
the gene-deficient microorganisms are produced by expressing a DNA  
section or a part-sequence thereof in the form of antisense RNA in  
microorganisms.

28. A method as claimed in claim 27,  
characterized in that  
the CAI method is used.

29. A method as claimed in <sup>claim 22</sup> ~~any of claims 22 to 28~~,  
characterized in that  
test systems selected from *in-vitro* systems, cell culture systems , tissue  
culture systems and animal models are used as natural environment to  
determine the viability or/and survivability of the gene-deficient  
microorganisms.

30. A method as claimed in claim 29,  
characterized in that  
the deficient gene sequences which lead to gene-deficient microorganisms  
which are not culturable and incapable of survival in the natural environment  
are assigned to the category of obligately essential genes.

31. A method as claimed in claim 29,  
characterized in that  
the deficient gene sequences which lead to gene-deficient microorganisms  
which are culturable but incapable of survival in the natural environment are  
assigned to the category of facultatively essential genes.

32. A method as claimed in <sup>claim 22</sup> ~~any of claims 22 to 31~~,  
characterized in that

the identification of the protein-encoding DNA section takes place by expression in a host organism and detection of the presence of an expression product.

- 5 33. <sup>claim 22</sup> A method as claimed in ~~any of claims 22 to 32,~~  
characterized in that  
it additionally comprises:
- (v) production of primers for amplification and detection of homologous gene sequences in heterologous microorganisms
  - 10 (vi) identification of the homologous gene sequences.

34. A nucleic acid coding for an essential secretory gene from *Helicobacter*,  
identified by the method as claimed in ~~any of claims 22 to 33.~~

15 35. A nucleic acid as claimed in claim 34,  
characterized in that  
it codes for a secreted polypeptide with signal peptide.

20 36. A nucleic acid as claimed in claim 34,  
characterized in that  
it codes for a secreted polypeptide without signal peptide.

25 37. A nucleic acid  
characterized in that  
it comprises

- (a) one of the nucleic acid sequences depicted in SEQ ID NO: n, where n is an odd integer from 1 to 245 inclusive, or a protein-encoding section thereof,
- (b) a nucleotide sequence corresponding to one of the sequences from (a) within the scope of the degeneracy of the genetic code or
- 30 (c) a nucleotide sequence hybridizing with one of the sequences from (a) and/or (b) under stringent conditions.

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38. A gene library comprising at least two nucleic acids as claimed in ~~any of~~ *claim 34*  
claims ~~34 to 37~~.

39. A vector  
5 characterized in that *claim 34*  
it comprises at least one nucleic acid as claimed in ~~any of claims 34 to 37~~ or  
a section thereof.

40. A vector as claimed in claim 39,  
10 characterized in that  
it is a CAI vector.

41. A vector as claimed in claim 39,  
15 characterized in that  
it is an SRM vector.

42. A cell,  
characterized in that  
20 it is transformed with a nucleic acid as claimed in any of claims 34 to 37 or  
a vector as claimed in any of claims 39 to 41.

43. A mutant library  
characterized in that  
25 it consists of at least two microorganisms transformed with a vector as  
claimed in claim 40 or with a vector as claimed in claim 41.

44. A polypeptide  
characterized in that  
30 it is encoded by a nucleic acid as claimed in *claim 34* ~~any of claims 34 to 37~~.

45. A polypeptide as claimed in claim 44,  
characterized in that

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it comprises

- (a) one of the amino acid sequences depicted in SEQ ID NO: m, where m is an even integer from 2 to 246 inclusive, or
- (b) a sequence which cross-reacts immunologically with one of the sequences according to (a).

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46. A polypeptide as claimed in claim 45,  
**characterized in that**  
it is an essential secreted polypeptide.

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47. A polypeptide fragment,  
**characterized in that**  
it has an immunogenic section of one of the sequences claimed in claim 45.

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48. An inhibitory molecule obtainable by the method as claimed in claim 1,  
**characterized in that**  
it is able to bind specifically to a polypeptide or fragment thereof as claimed in any of claims 44 to 47 or/and to influence the expression, presentation or/and natural function thereof.

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49. A method for producing a polypeptide or polypeptide fragment as claimed in any of claims 44 to 47

**characterized in that**

a cell is transformed with a nucleic acid as claimed in any of claims 34 to 37 or with a vector as claimed in claim 39, the transformed cell is cultivated under conditions with which expression of the polypeptide takes place, and the polypeptide is isolated from the cell or/and the culture supernatant.

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50. The use of a polypeptide or of a fragment thereof as claimed in <sup>claim 44</sup> ~~any of claims 44 to 47~~ thereof as immunogen for generating antibodies.

30

51. An antibody or fragment thereof,

**characterized in that**

it is specific for a polypeptide or a fragment thereof as claimed in any of claims 44 to 47.

- 5 52. A pharmaceutical composition,  
**characterized in that**  
it comprises as active ingredient
- a) a nucleic acid as claimed in any of claims 34 to 37,
  - b) a vector as claimed in claim 39,
  - 10 c) a cell as claimed in claim 42,
  - d) a polypeptide or a fragment thereof as claimed in any of claims 44 to 47,
  - e) an antibody or fragment thereof as claimed in claim 51 and/or
  - f) an inhibitory molecule as claimed in claim 48
- where appropriate together with conventional pharmaceutical excipients,  
15 diluents, additives and carriers.

53. The use of a pharmaceutical composition as claimed in claim 52 for the  
diagnostic, prevention or/and therapy of a *Helicobacter* infection.

20 54. The use of a pharmaceutical composition as claimed in claim 52 for  
inhibiting the reproduction of *Helicobacter* organisms and/or other  
anthrogonic microorganisms in a host.

25 55. The use as claimed in claim 54,  
**characterized in that**  
a nucleic acid as claimed in any of claims 34 to 37 is formulated as DNA  
vaccine.

30 56. The use as claimed in claim 54,  
**characterized in that**  
a polypeptide or polypeptide fragment as claimed in any of claims 44 to 47  
is formulated as subunit vaccine or as live vaccine.

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57. The use of a pharmaceutical composition as claimed in claim 52 for producing an agent for the diagnostic, prevention or/and therapy of a *Helicobacter* infection.

5 58. A vector as claimed in claim 41,  
characterized in that  
the SRM vector is the vector pSRM4 (SEQ ID No. 247).

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